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PLANT EXTRACTS ALONG WITH SELECTIVE CHEMICALS AND *Bacillus thuringiensis israelensis*: A NOVEL APPROACH TO TACKLE THE PROBLEM OF INSECTICIDAL RESISTANCE IN MOSQUITOES

Imran Ahmed[1], Shabab Nasir[1,*], Iqra Yousaf[1], Bilal Ahmad[1], Sumbal Zafar [1] and Aqib Javaid[1]

[1]Department of Zoology, Government College University, Faisalabad-38040, Punjab, Pakistan
[*]Corresponding author's e-mail: flourenceshabab@yahoo.com

Mosquitoes act as vectors for many life-threatening diseases such as malaria, dengue fever, West Nile virus, zika virus etc. Continuous use of pesticides caused insecticidal resistance in mosquitoes along with many other detrimental effects on the environment. Therefore, this study was designed to manage the mosquito population under laboratory conditions with synthetic insecticides, plant extracts, and *Bacillus thuringiensis israelensis* (Bti). Mosquito larvae (*Aedes aegypti*) were collected, identified and reared in cages for bioassay. Along with synthetic chemicals and Bti, fifteen plants were selected for this study and their extracts were obtained through ether as a solvent. Two concentrations (0.001 and 10%) of each treatment were applied against 2nd and 3rd instar larvae for screening trails. Data were collected to check knockdown effect after 4 and 64 h and analysed through two-level factorial design. Datura (*Datura wrightii*) leaf, kortumma (*Citrullus colocynthis*), chirata (*Swertia chirayaita*), *Bti*, deltamethrin and mortein liquid was screened out as significant factors. After screening experiments, significant factors were tested in combination with five concentrations (0.001, 0.01, 0.1, 1.0 and 10%) of each treatment to test their efficacy after 2, 4, 8, 16, 32, 64 and 128 h. In the mixing trials, the highest mortality was observed with those solution having kortumma, insecticides and Bti whereas the least value of LC_{50} (45.2%) with 100% mortality was found in combination of Kortumma, Datura leaf, Bti, deltamethrin and mortein liquid. Plant extracts along with chemicals and Bti can be considered as an alternative strategy for the control of mosquitoes.

Keywords: Mosquito-borne diseases, dengue fever, *Aedes aegypti*, mortality, plant extracts, resistance management.

INTRODUCTION

Mosquitoes are vector of many fatal diseases like malaria, dengue, zika virus, West Nile virus, and filariasis (Ghosh *et al*., 2012). There are different kinds of mosquitoes in the world but *Anopheles*, *Aedes*, and *Culex* species are medically important (Rathy *et al*., 2015). *Aedes* mosquitoes are important vectors of dengue fever, lymphatic filariasis, chikungunya, yellow fever, and Zika virus as there are more than 50 million cases of dengue infection worldwide each year (Sinha *et al*., 2004). Dengue hemorrhagic fever affects about 500,000 patients worldwide (WHO, 2011). As mosquito-borne diseases are mostly caused by virus, so, there is neither a proper vaccine nor treatment available for most of these diseases. The management of mosquitoes through insecticides does not only cause resistance development but also the biological magnification along with bad impact on the environmental quality (Ahmad *et al*., 2018). Because of these substances, the human body immune system becomes weak against the diseases (Sinha *et al*., 2004). Therefore, alternative methods of control are needed (Borum and Gunes, 2018). For this purpose, a new strategy of Integrated Mosquito Management (IMM) is introduced for the management of mosquitoes. For IMM, alternative strategies like public awareness, mosquito surveillance, source reduction, and eco-friendly least-toxic larval control are promoted. Researchers have urged to use eco-friendly, biodegradable, and target oriented insecticides against mosquitoes (Naseem *et al*., 2018). Taking these points into consideration, alternative strategies such as the use of Insect Growth Regulators (IGR.s), plant extracts and *Bacillus thuringiensis israelensis* (Bti) are given importance. Plant extracts are mixtures of different chemical compounds such as terpenoids, alkaloids, steroids, phenols, flavonoids, tannins, and saponins that exhibit ovicidal, larvicidal, mosquitocidal and synergistic properties (Gurudeeban *et al*., 2010). As they do not develop resistance and safe for the environment (Ghosh *et al*., 2012). Bti is very effective for the control of mosquito larvae and some other dipterans. These bacterial agents have been used as larvicides and called as biocides or biolarvicides. These biolarvicides are greatly effective due to the production of toxins in the gut of mosquito larvae even at very low doses and totally safe to other non-target organisms (Chang *et al*., 2014).

In Punjab Province, *Culex*, *Anopheles* and *Aedes* are commonly found almost year-round. Among these, *Anopheles* spp. do not only cause a biting nuisance in summer months but also a serious threat to public health due to their potential for malaria transmission. About a quarter of Punjab population is at the potential risk of Malaria infection (Qasim

et al., 2014), as Punjab is the most populated province of Pakistan. Since 2010, dengue outbreaks occur every year in Punjab during the rainy season when the conditions are conducive for mosquito breeding. Dengue fever was first reported during 1994 and a huge epidemic occurred in Punjab province during 2011 with 100 confirmed cases daily (Shakoor *et al*., 2012). Punjab is an agricultural province and different types of trees, herbs, and shrubs are grown here. These belong to different plant families such as Solanaceae, Meliaceae, Asteraceae, Cucurbitaceae, Gentianaceae, Euphorbiaceae, Zingiberaceae etc that have antimicrobial, antifungal, anti-inflammation and different types of larvicidal or repellent activities against various species of mosquitoes (Shaalan *et al*., 2005). Contrary to this fact, blindly use of insecticides to save the crops also causes insecticidal resistance in mosquitoes too (Ahmad *et al*., 2002). So, this study is proposed to evaluate the extracts of our local weeds, herbs, and shrubs in combination with Bti and growth regulators.

MATERIALS AND METHODS

Mosquito larvae collection and rearing: Mosquito (*Ae. aegypti*) larvae collected with a dipper from different residential sites (geographic coordinates: 31°26′2.18″N 73°3′53.6″E) were brought to the entomology laboratory in the Department of Zoology, Government College University Faisalabad, Pakistan for rearing (Nasir *et al*., 2017a). After rearing in cages (2 x 2 x 2 feet), the adults were identified using standard manual (Qasim *et al.*, 2014). Male mosquitoes were fed 10% sugar solution while female with rat blood (Nasir et al., 2017a). After egg laying, hatched larvae (2nd and 3rd) were used for this study. Twenty larvae of both groups were placed into beakers containing 250 ml of water along with fish diet.

***Collection of plant samples and handling*:** Plant samples were selected based on their local availability and insecticidal properties (Gurudeeban *et al.*, 2010). The plant parts were collected from healthy plants, free from dust, dirt and other impurities. After washing, the material was dried under shade and then oven dried (Nasir et al., 2017b). Dried Plant material was transferred into powdered form by using an electrical grinder (Kenwood BL-480). Then this powder was used for aqueous and oil solution extraction.

***Oil extraction*:** Essential oils were extracted from the selected plant parts with the help of Soxhlet apparatus (Cheng *et al*., 2009) by using ether as a solvent.

Synthetic insecticides: Temephos, Deltamethrine, Hexane, Altosid, Mortein liquid, Nemokill and Bti were used for this study (Table 2).

***Screening of plant extracts and commercial insecticides*:** During screening trials, ether extracts of fifteen plants (Table 1), six chemicals (Table 2) and Bti were evaluated for their efficacy in controlling mosquito larvae. These trials were conducted by using two concentrations, lower (0.001%) and upper (10%) in three replications of each treatment along with control group and mortality was observed after 4 and 64 h (Ahmad *et al*., 2017).

Mixing treatments: After screening trials, significant factors were evaluated against mosquito larvae in different sixty three (2^6 where 6 is significant factors during screening trails) combinations (mixture of two, three, four, five and six factors) separately. Five concentrations (0.001, 0.01, 0.1, 1.0 and 10%) of each combination in 3 replications were used along with the control group. Mortality data was calculated to check the knockdown effect after 4, 8, 16, 32, 64 and 128 h.

Data collection: Mortality data was collected. All the results were recorded after the interval of 4 and 64 h for screening trials and 4, 8, 16, 32, 64 and 128 h for mixing trials.

Table 1.Name of the potent plant used for control of mosquito genera.

Plant local name	Plant scientific name	Family	Order	Part used
Neem	*Azadirachta indica* A. Juss	Meliaceae	Sapindales	Leaf
Kasni	*Cichorium intybus* L.	Asteraceae	Asterales	Leaf
Datura	*Datura wrightii* Regel.	Solanaceae	Solanales	Leaf
Datura	*Datura stramonium* L.	Solanaceae	Solanales	Fruit
Aak	*Calotropis procera* Aiton	Apocynaceae	Gentianales	Leaf
Karela	*Momordica charantia* L.	Cucurbitaceae	Cucurbitales	Fruit
Amer bel	*Cuscuta pentagona* Englem.	Convolvulaceae	Solanales	Plant
Bathua	*Chenopodium album* L.	Amaranthaceae	Caryophyllales	Leaf
Kortumma	*Citrullus colocynthis* L.	Cucurbitaceae	Cucurbitales	Fruit
Chebbr	*Melothria scabra* Naudin	Cucurbitaceae	Cucurbitales	Fruit
Chirata	*Swertia chirayaita* H. Karst	Gentianaceae	Gentianales	Fruit
Hernoli	*Ricinus communis* L.	Euphorbiaceae	Malpighiales	Leaf
Bhang	*Cannabis sativa* L.	Cannabaceae	Rosales	Plant
Adrak	*Zingiber officinale* Roscoe	Zingiberaceae	Zingiberales	Corm
Neem Seeds	*Azadirachta indica* A. Juss	Meliaceae	Sapindales	Seed

Table 2. Chemical used as insecticides.

Chemicals	Trade mark	Chemical group	Active ingredient	Manufacturer country
Temephos	Abate®	Organophosphate	1.1% w/w	Malaysia
Deltamethrin	Deltamethrin 2.5 EC	Pyrethroid	2.5% w/v	China
Hexane	N Hexane	Organic compound	68%	China
Altosid	Altosid® Pro G	Methoprene	1.5%	USA
Mortein liquid	Mortein®	Pyrethroid	Allethrin (2.09 g/kg) and Resmethrin (0.39 g/kg)	Australia
Neem oil	Neemkill	Plant extract	Neem oil	India

***Phytochemical components of significant plant extracts*:** Qualitative phytochemical tests were carried out on the plant extracts according to the standard procedure described by Bargah (2015).

Analysis of data: The collected data was corrected through Abbott's formula (Abbot, 1925) and then was subjected to two-level factorial design for screening of the significant factors from twenty two initial factors by using Design-Expert® software version 8. For identified significant factors and their combinations, MS Excel 2007 was used to find the mean and standard deviation, LC_{50} and LT_{50} using probit analysis with Minitab 17 (Cheng *et al.,* 2009b).

RESULTS

Significant factors contributing for mortality were screened out from fifteen plant extracts, Bti and six chemicals (Table 3). Data regarding mortality in case of screening trails were analyzed through two level factorial design to screen out significant factors responsible for mortality in terms of F and p-values (Table 3). Datura leaf, kortumma fruit, chirata, *Bti*, deltamethrine and mortein liquid were found significant in case of causing mortality and p-value as shown in Table 3.

In case of significant single factors, more than 55% mortality was observed after 32 hfor every single factor as shown in Table 4. Mortality recorded in case of chemicals was 76.9% followed by plant extracts (68.2%) and then Bti (57.5%) with LC_{50} value (188.4 ppm) as shown in table 4.In case of two factors combination, some combinations antagonist the effect of their partner component (kortumma alone caused 68% mortality while in combination caused 65 and 55% mortality with datura leaf and chirata respectively) and some synergist

Table 3. p-value for mortality under different screening designs for main-effects model.

Sources	df	2-level factorial		Sources	df	2-level factorial	
		F-value	p-value			F-value	p-value
Model	21	78.16	<0.0001**	N-Bhang plant	1	2.98	0.4531
A-Neem leaf	1	1.34	0.1105	O-Zinger plant	1	6.12	0.3471
B-Kasni leaf	1	141.37	0.4914	P-Neem seeds	1	3.57	0.4213
C-Datura leaf	1	70.86	<0.0001**	Q-Temephos	1	8.67	0.5231
D-Datura fruit	1	7.44	0.3210	R-Deltamethrin	1	82.98	<0.0001**
E-Akk leaf	1	0.34	0.4514	S-Hexane	1	76.87	0.4532
F-Karela fruit	1	0.0043	0.8780	T-Altosid	1	2.14	0.6534
G-Amber plant	1	5.83	0.0862	U-Mortein liquid	1	13.78	0.0041*
H-Bathua leaf	1	2.58	0.1191	V-Neemkill	1	3.78	0.4325
J-Kortumma fruit	1	11.37	0.0110*	W-*Bti*	1	10.09	<0.0130*
K-Chebber fruit	1	0.51	0.0901	Residual	501		
L-Chirata plant	1	9.34	0.0162*	Cor. Total	522		
M-Hernoli leaf	1	3.67	0.3290				

*means significant at p<0.05; **means highly significant at p<0.001

Table 4. Percent mortality and LC_{50} (Mean ± SE) of single significant factors after 32 hours against *Aedes aegypti* larvae.

Single factors	% mortality at 0.01 % con. ±SE	LC_{50} ±SE	FL at 95% C.I
Kortumma	68.17±0.095	125.22±0.014	115.14-131.25
Datura leaf	66.61±0.113	133.63±0.035	125.35-142.33
Chirata	63.78±0.223	144.12±0.116	135.65-162.47
Bti	57.50±0.000	188.41±0.039	178.22-197.20
Deltamethrin	76.94±0.747	168.50±0.054	152.14-186.33
Mortein liquid	73.94±0.310	155.35±0.066	147.31-164.36

the effect of their partner (combinations of plant extracts with chemicals caused more than 70% mortality) as shown in Table 5. Moreover, the highest mortality (96%) was seen in combination of both chemicals (deltamethrin and mortein liquid) followed by the combination of datura leaf and deltamethrin (91.67%) and least mortality (66%) was seen in case of chirata and Bti. In case of LC_{50}, combinations having plant extracts had greater values (more than 100%) as compared to other combinations (less than 100%) as shown in Table 5.

In case of three factors combination, all the combinations showed more than 80% mortality, whereas the total mortality rate was observed in combination of Bti, deltamethrine and mortein liquid with least LC_{50} value (47.3%) while least mortality (68%) was observed in case of kortumma, datura leaf and chirata with 145% LC_{50} value as shown in Table 6. In case of four factors combination, all the combinations showed more than 95% mortality at 0.01% concentration after 32 h. The total mortality rate was observed in combination of Kortumma, Bti, deltamethrine and mortein liquid with least

Table 5. Percent mortality and LC_{50} (Mean ± SE) in combination of two significant factors after 32 hours against *Aedes aegypti* larvae.

Two factors combination	% mortality at 0.01 % con. ±SE	LC_{50}±SE	FL at 95% C.I
Kortumma + Datura leaf	65.00±0.013	147.02±0.016	141.41-153.25
Kortumma + Chirata	55.00±0.015	155.01±0.022	147.25-163.22
Kortumma + Bti	75.00±0.017	141.14±0.072	130.56-170.11
Kortumma + Deltamethrin	90.00±0.031	108.25±0.077	99.37-115.75
Kortumma + Mortein liquid	85.00±0.041	105.12±0.025	94.61-120.20
Datura leaf + Chirata	73.00±0.121	154.02±0.030	143.26-169.69
Datura leaf + Bti	72.67±0.100	121.01±0.004	112.29-131.55
Datura leaf + Deltamethrin	91.67±0.100	103.14±0.041	91.31-118.50
Datura leaf + Mortein liquid	88.00±0.021	101.10±0.020	88.85-114.10
Chirata + Bti	66.00±0.122	135.00±0.003	122.20-146.11
Chirata + Deltamethrin	88.33±0.100	132.12±0.056	120.52-147.36
Chirata + Mortein liquid	85.00±0.034	125.10±0.033	115.08-136.33
Bti + Deltamethrin	75.00±0.032	66.53±0.380	56.25-77.650
Bti + Mortein liquid	73.00±0.123	99.20±0.510	92.35-108.23
Deltamethrin + Mortein liquid	96.00±0.221	47.30±0.330	43.22-56.340

Table 6. Percent mortality and LC_{50} (Mean ± SE) in combination of three significant factors after 32 hours against *Aedes aegypti* larvae.

Three factors combination	% mortality at 0.01 % con. ±SE	LC_{50}±SE	FL at 95% C.I
Kortumma + Datura leaf + Chirata	68±0.012	145.00±0.010	138.25-152.47
Kortumma + Datura leaf + Bti	72±0.032	78.04±0.019	65.56-85.740
Kortumma + Datura leaf + Deltamethrin	95±0.019	99.63±0.534	88.46-108.40
Kortumma + Datura leaf + Mortein liquid	91±0.021	96.52±0.234	85.38-110.15
Kortumma + Chirata + Bti	75±0.096	95.00±0.012	87.21-104.11
Kortumma + Chirata + Deltamethrin	95±0.054	101.43±0.050	92.65-111.01
Kortumma + Chirata + Mortein liquid	90±0.121	99.21±0.034	80.21-109.35
Kortumma + Bti + Deltamethrin	90±0.092	57.46±0.840	49.32-65.390
Kortumma + Bti + Mortein liquid	86±0.076	59.42±0.540	52.01-64.620
Kortumma + Deltamethrin + Mortein liquid	90±0.023	98.24±0.079	90.20-105.40
Datura leaf + Chirata + Bti	75±0.012	93.00±0.010	85.43-99.090
Datura leaf + Chirata + Deltamethrin	100±0.010	121.35±0.140	115.13-130.48
Datura leaf + Chirata + Mortein liquid	98±0.020	116.31±0.090	108.47-125.11
Datura leaf + Bti + Deltamethrin	100±0.010	67.37±0.440	60.36-72.250
Datura leaf + Bti + Mortein liquid	95±0.020	70.20±0.260	65.25-77.960
Datura leaf + Deltamethrin + Mortein liquid	91±0.020	94.13±0.049	86.36-103.50
Chirata + Bti + Deltamethrin	100±0.010	71.15±0.560	65.35-76.520
Chirata + Bti + Mortein liquid	95±0.020	74.74±0.130	68.19-79.200
Chirata + Deltamethrin + Mortein liquid	88±0.120	90.12± 0.05	82.47-126.36
Bti + Deltamethrin + Mortein liquid	100±0.010	47.30±0.330	40.28-55.250

Table 7. Percent mortality and LC_{50} (Mean ± SE) in combination of four significant factors after 32 hours against *Aedes aegypti* larvae.

Four factors combination	% mortality at 0.01 % con. ±SE	LC $_{50}$±SE	FL at 95% C.I
Kortumma + Datura leaf + Chirata + Bti	90±0.12	92.02±0.01	86.33-97.25
Kortumma + Datura leaf + Chirata + Deltamethrin	100±0.01	88.85±0.87	80.43-96.57
Kortumma + Datura leaf + Chirata + Mortein liquid	95±0.02	85.76±0.55	79.41-96.58
Kortumma + Chirata + Bti + Deltamethrin	100±0.01	78.50±0.26	69.87-88.63
Kortumma + Chirata + Bti + Mortein liquid	98±0.02	80.25±0.22	71.35-93.65
Kortumma + Chirata + Deltamethrin + Mortein liquid	95±0.12	94.41±0.09	85.25-100.7
Kortumma + Datura leaf + Bti + Deltamethrin	100±0.00	89.67±0.22	81.77-96.80
Kortumma + Datura leaf + Bti + Mortein liquid	95±0.10	90.52±0.22	82.45-105.7
Kortumma + Datura leaf + Deltamethrin + Mortein liquid	95±0.11	89.67±0.22	76.68-96.65
Datura leaf + Chirata + Bti + Deltamethrin	100±0.00	64.85±0.71	58.62-75.45
Datura leaf + Chirata + Bti + Mortein liquid	95±0.04	66.45±0.63	58.40-80.33
Datura leaf + Chirata + Deltamethrin + Mortein liquid	100±0.01	82.33±0.15	75.69-117.6
Datura leaf + Bti + Deltamethrin + Mortein liquid	100±0.00	67.37±0.44	59.60-74.25
Chirata + Bti + Deltamethrin + Mortein liquid	100±0.00	82.15±0.56	77.20-87.74
Kortumma + Bti + Deltamethrine + Mortein liquid	100±0.00	57.46±0.84	52.66-66.31

Table 8. Percent mortality and LC_{50} (Mean ± SE) in combination of five and six significant factors after 32 hours against *Aedes aegypti* larvae.

Five and six factors combinations	% mortality at 0.01 % con. ±SE	LC_{50}±SE	FL at 95% C.I
Kortumma + Datura leaf + Chirata + Bti + Deltamethrin	100±0.01	71.28±0.65	66.82-76.96
Kortumma + Datura leaf + Chirata + Bti + Mortein liquid	100±0.00	73.14±0.47	68.20-78.25
Kortumma + Datura leaf + Bti + Deltamethrin + Mortein liquid	100±0.01	45.21±0.79	35.29-56.45
Kortumma + Chirata + Bti + Deltamethrin + Mortein liquid	100±0.01	78.50±0.26	71.11-85.69
Datura leaf + Chirata + Bti + Deltamethrin + Mortein liquid	98.3±0.01	84.85±0.71	58.54-99.99
Kortumma + Datura leaf + Chirata + Deltamethrin + Mortein liquid	100±0.0	78.85±0.86	71.35-96.46
Kortumma + Datura leaf + Chirata + Bti+Deltamethrin + Mortein liquid	100±0.0	71.28±0.65	66.36-78.06

LC_{50} value (57.46%) while the least mortality (90%) was observed in case of kortumma, datura leaf, chirata and Bti with 92.01% LC_{50} value as shown in Table 7.

In case of five and six factors combinations, all the combinations except one (datura leaf, chirata, Bti, deltamethrin and mortein liquid) gave 100 % mortality at 0.01 % concentration after 32 h as shown in Table 8. The most potent combination was that of kortumma, datura leaf, Bti, deltamethrin and mortein liquid with least LC 50 value (45.21%).

Table 9 shows that flavonoids were present in kortumma and chirata plant extracts through ether while alkaloid is present in the chirata and neem leaf plant extracts. Saponin was mildly present in kortumma. Tannins were highly present in kortumma, chirata and neem leaf. Anthranoids and phlobatanins were not detected in the plant extracts through ether as shown in the table.

Table 9. Phytochemical components of significant plant extracts through ether.

Components	Kortumma	Datura leaf	Chariata
Saponin	+	-	+
Alkaloids	-	+	++
Phenols	+	-	+
Tannins	+++	-	+
Cardiac glycosides	+	-	-
Terpenes	++	++	+
Steroids	++	+++	++
Flavonoids	+++	+	+++
Anthraquinones	+	+	+
Anthranoids	-	-	-
Phlobatanins	-	-	-

+++: Highly present ++: Moderately present +: Mildly present
- : Not detected

DISCUSSION

This study compared the biological and chemical control against *Ae. aegypti* mosquito with the use of plant extracts and *Bti*. This study highlighted the potential of plants, insecticides and *Bti* for mosquito control and the prevention of dengue and malarial vectors. Thus, this study underlined several important entomological parameters that should be quantified to determine the impact of plants, insecticides and *Bti*. In single trials, three plant extracts (ether) were used against dengue larvae. A higher mortality rate was observed in case of kortumma, and datura leaf extracts than the chariata and *Bti*. Highest mortality (76%) was observed in case of deltamethrin and least 57% was observed in case of *Bti* for larvae of *Ae. aegypti*. The Kortumma and datura leaf extracts through ether showed the 68.17 and 66.61% mortality, respectively. These results are in line with the results of various other researchers who also reported botanicals as effective mosquito larvicides (Ghosh *et al*., 2012; Raveen*et al*., 2014; Nasir *et al*., 2017b). The obtained results showed that 68% mortality in case of *C. colocynthis* extract treatment. These were consistent with the results of Ghosh *et al*. (2012) who observed the 70% mortality in case of *C. colocynthis* plant extract. In screening trials with synthetic insecticides, more than 70% mortality was observed with deltamethrin and mortein liquid were found more effective against mosquito larvae. These findings agreed with the other researchers who also reported more than 85% mortality with deltamethrin against *Ae. aegypti* (Gurudeeban *et al*., 2010). In case of two factors combined, the highest mortality 75% was seen with plant extract of kortumma and Bti after 32 h. These results were in the agreement with Prabhu *et al*. (2011) who reported the efficacy of *Moringa oleifera* seed extracts with Bti against malarial vector (*Anopheles stephensi*) as more than 90% reduction in larvae after 72 h. Chang *et al*. (2014) and Kumar *et al*. (2012) also found binary mixtures of plant extracts and Bti more toxic against mosquito larvae as compared to the plant extracts and Bti separately. Bti with insecticides proved themselves as highly toxic to mosquito larvae and this response was time and concentration dependent in all larval stages. Our results showed higher mortality in case of combined mixture of Bti and deltamethrin as compared to the alone against mosquito larvae. These results are in line with Tetreau *et al*. (2013) who also observed a higher toxicity (over 6·72-fold) of the composite deltamethrin-Bti towards mosquito larvae as compared to Bti alone. The combination of plant extracts with insecticides proved themselves as highly toxic to mosquito larvae and this response was time and concentration dependent in all larval stages. The obtained results after 32 h showed that highest mortality was seen in all the types of plant extracts mixed with insecticides by using 0.01% concentration of solutions. These results are also in line with Elango *et al*. (2011) who found that the plant extracts with insecticides 100% larval mortality in methanol extract of *A. lineata*, and *C. indium* after 24 h and the hexane extract of *A. lineata* and *D. metal* after 48 h at 10 mg/100 ml. These results agree with other researchers who found plant extracts and chemicals in combination ratios 1:1 more effective than other ratios with LC_{50} values (0.0013, 0.0010 and 0.0009 mg/L) and (0.0016, 0.0014 and 0.0013 mg/L) against *anopheline* and *culicine* larvae after 24, 48 and 72 h of treatment, respectively (Bhan *et al.,* 2015). These findings are also in agreement with the Raghavendra *et al*. (2013) who observed two plant extracts along with deltamethrin synergistic efficacy against *Aedes aegypti* larvae. The deltamethrin when analyzed separately, LC_{50} and LC_{90} values were 0.00045 and 0.00148 ppm, respectively. Synergistic studies with two plant extracts on deltamethrin revealed *S. canadensis* as more effective with synergistic factor (SF) of 4.090 for LC_{50} value followed by *E. jambolana* with SF 1.80 for LC_{50} at 1:1 ratio of the plant extracts and deltamethrin.

***Conclusion*:** Use of different combinations of plant extracts and insecticides with *Bti* as mentioned in this work had been proved to be effective and safer for the control of dengue vector. Some combinations were synergists in their action, so we should use different plant extract mixtures with Bti to get maximum mortality instead of using synthetic insecticides. Moreover, biopesticides could serve as a good eco-friendly and cost-effective approach to reduce the dose of chemicals with high residual effect to be applied in vector control programs.

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